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Filed : **March 16, 2007**

REMARKS

Claims 1, 4, 5, 7 and 8 have been amended. Claims 2 and 3 have been canceled. New claim 30 has been added. Claims 20-29 have been withdrawn from consideration as being directed to a non-elected invention. Thus, claims 1 and 4-30 are now pending in the present application, with claims 1, 4-19 and 30 currently under consideration. Support for the amendment to claim 1 may be found in original claims 2 and 3. No new matter has been added. Reconsideration and withdrawal of the present rejections in view of the comments presented herein are respectfully requested.

Rejections under 35 U.S.C. § 103(a)

Claims 1, 3-5 and 8-13 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Dean et al. (*Proc. Natl. Acad. Sci. USA* 99:5261-5266, 2002) in view of Berlin (US 7,008,770) and further in view of Olek (*Nucl. Acids Res.* 24:5064-5066, 1996) (“combination 1”).

Claim 2 was rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the combination 1, and further in view of Raizis et al. (*Anal. Biochem.* 226:161-166, 1995).

Claims 6 and 7 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over combination 1, and further in view of Christensen et al. (US 2006/0014144).

Claims 14-19 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over combination 1, and further in view of Hogrefe et al. (US 2002/0143577).

The Examiner alleges that it would have been obvious to combine the whole genome amplification methods of Dean and Berlin using nuclease-resistant hexamer primers to amplify genomic DNA by multiple displacement amplification (Dean) and amplifying bisulphite-treated DNA using primers containing 3 of 4 bases (Berlin) with the methods of Olek, who teaches sodium bisulphite treatment of DNA embedded in agarose to from and maintain the DNA in single-stranded form during treatment, since the methods of bisulphite treatment of Berlin and Olek can be easily adapted to the methods of Dean with appropriately designed primers to amplify bisulphite-modified templates. Raizis et al. was cited for its teaching of a DNA bisulphite treatment method that minimizes template degradation, Christensen for its teaching of oligonucleotides containing intercalator analogs, and Hogrefe for its teaching of high fidelity

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DNA polymerase compositions. However, as explained below, none of these combinations of references would render the presently claimed invention obvious.

As noted in the enclosed Rule 132 Declaration of Dr. Douglas Spencer Millar (paragraph 6), the presently claimed method relates to treatment of genomic DNA with an agent which modifies cytosine bases, but does not modify 5'-methyl-cytosine bases, prior to amplification. This treatment effectively modifies the whole genome of the organism being investigated so that all unmethylated cytosines are converted, via uracil, to thymine and thus the modified genome comprises predominantly only 3 bases (A, G and T) instead of the native 4 bases (A, C, G and T). Modifying the genome in such a manner can be particularly advantageous in certain circumstances, for example when examining the genome for methylation analysis, in which the only cytosines present in the modified genome are methylated cytosines. This method allows amplification of bisulphite, acetate or citrate-treated genomic DNA without substantial fragmentation, which facilitates the generation of long amplification products (for example, >20 kb in size), and allows amplification of such treated DNA from as little as 10 cells (Declaration, paragraph 7). This provides significant advantages over the methods disclosed in the cited references.

Dean et al. teaches a method for multiple displacement amplification of native genomic DNA using phi29 DNA polymerase and exonuclease resistant primers. However, the method of Dean cannot be used for methylation analysis, as there is no distinction between methylated and non-methylated cytosines (Declaration, paragraph 8).

Olek et al teaches a method for shearing or digesting genomic DNA, treating the sheared DNA with a bisulphite reagent, amplifying the treated DNA and analyzing the amplified DNA. In the method of Olek, the DNA is sheared or digested prior to bisulphite treatment, and thus results in amplification and analysis of relatively small (<2 kb) modified genomic DNA fragments. In contrast, the present method is used to treat whole, undigested or unsheared, genomic DNA with a bisulphite, acetate or citrate modifying agent, which converts the unmethylated cytosine bases to thymines, without substantial DNA fragmentation (Declaration, paragraph 7). The present method unexpectedly and advantageously permits analysis of whole chemically converted genomic DNA, and of amplification products >20 kb in length, from the treated genomic DNA, which provides a clear and useful advantage compared to the method of Olek et al. (Declaration, paragraph 9).

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Berlin provides a method for performing complex PCR amplification following treatment of genomic DNA with sodium bisulphite which could be used for methylation analysis. However, as indicated by the Examiner, a preferred method of bisulphite treatment for preparation of DNA for PCR and methylation analysis is the method of Olek, and as outlined above, the present invention is clearly advantageous over that method. The fact that a minimum of 50 type 1 primers directed towards one strand of DNA is required to successfully amplify a large portion of the genome is further evidence that the methods used by Berlin result in fragmentation of the DNA and thus amplification of relatively small modified genomic DNA fragments (Declaration, paragraph 10).

Raizis teaches a method for bisulphite treatment of DNA that minimizes template degradation, including reduction of the time required to achieve completion of the bisulphite reaction using higher concentrations of bisulphite, compared to traditional methods. Raizis demonstrates amplification of a minimum of 1 pg of plasmid DNA of 6930 bp in size (5790 bp for the PGL2-Promoter plasmid plus 1140 bp neomycin gene), which is equivalent to 1.34×10^5 copies of plasmid per PCR (Declaration, paragraph 11). In contrast, the presently claimed invention, allows amplification of bisulphite, acetate or citrate-treated DNA from as little as 10 cells (Declaration, paragraph 7) which is a vast improvement over the method of Raizis, and indicates that the present invention almost completely eliminates template degradation, and therefore provides an unexpected advantage over Raizis.

The inability of Raizis to amplify from less than 100,000 copies of plasmid indicates that although their method may represent a significant improvement over traditional bisulphite methods, it still results in a significant amount of DNA degradation (Declaration, paragraph 11).

Conversion of DNA using traditional methods of bisulphite treatment, such as those used in Olek et al and Berlin, results in significant degradation of DNA with the concomitant loss of up to 96% of the starting DNA (Grunau et al 2001; *Nucleic Acids Res.* 29 (13); E65-5). As noted above, the presently claimed method allows complete conversion of DNA without substantial DNA fragmentation. This is evidenced by the ability to amplify fragments >20 kb at various loci throughout the converted genome and from as little as 10 cells (specification at page 51, lines 29-35), which is significantly better than the method of Raizis. The bisulphite conversion method taught in Olek et al requires more experimental steps and would result in loss of DNA. These unexpected advantages are neither disclosed nor suggested by the cited references, and could not

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have been predicted based upon these references. These unexpected results would effectively rebut any allegation of *prima facie* obviousness if one were present, and strongly support the nonobviousness of the present claims. The remaining references (Christensen et al and/or Hogrefe) do not teach or suggest reduction in substantial DNA fragmentation, and therefore do not teach nor suggest the unexpected advantages discussed above.

In view of the comments presented above, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a).

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

Applicants submit that all claims are in condition for allowance. However, if minor matters remain, the Examiner is invited to contact the undersigned at the telephone number provided below.

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Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated:

8/11/09

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